1. PURPOSE

1.1. To ensure the safety of people in the Microbiology laboratories at Thompson Rivers University (TRU).

2. SCOPE

2.1. This procedure applies to all employees and students at TRU.

3. PRECAUTIONS

POTENTIAL HEALTH & SAFETY HAZARDS

HAZARD		TO PROTECT YOURSELF
Fire		Know the location of fire alarm stations, fire extinguishers, and fire exits. Ensure fire extinguisher on hand is appropriate for fires that may occur within the facilities in question. Keep area around and near open flames in the lab clear of obstructions. Do not keep volatile solvents in open beakers. When manipulating flammable chemicals, do so in a certified, operating fume hood with the sash pulled down to a protective level. Turn off burners when not in use for aseptic technique and always ensure burner area is clear of clutter and any flammable and any other unnecessary chemicals. Always wear properly fitted and inspected TRU laboratory mandated personal protective equipment when using open flames or handling flammable materials: safety glasses or goggles, closed toed shoes, laboratory coat, and gloves. Wash hands before and after the use of disposable gloves. Respirators and heat resistant or other specialized hand protection use may be necessary for some heating protocols and other chemical manipulations. Keep workspace clear of unnecessary materials. Label all laboratory chemicals with appropriate hazard signage.
Explosion		Never heat a closed system. When heating of a potentially explosive chemical, do so in a certified, operating fume hood with the sash pulled down to a protective level. When heating a vessel with a closable lid is necessary, ensure the vessel lid is loosely closed to ensure building pressure can escape to the outside environment. Turn off burners when not in use for aseptic technique and always ensure burner area is clear of clutter and any hazardous or other unnecessary chemicals. Always wear properly fitted and inspected TRU laboratory mandated personal protective equipment if handling potentially explosive materials: safety glasses or goggles, closed toed shoes, laboratory coat, and gloves.

		Wash hands before and after the use of disposable gloves. Respirators and heat resistant or other specialized hand protection use may be necessary for some heating protocols and other chemical manipulations. Keep workspace clear of unnecessary materials. Label all laboratory chemicals with appropriate hazard signage.
Chemical or Thermal Burn		Always assume hot plates are hot. Many organic and inorganic chemicals are corrosive to the skin and eyes. When volatile or toxic chemicals/substance heating and/or manipulation is necessary, do so in a certified, operating fume hood with the sash pulled down to a protective level. Turn off burners when not in use for aseptic technique and always ensure burner area is clear of clutter and any hazardous or other unnecessary chemicals. Always wear properly fitted and inspected TRU laboratory mandated personal protective equipment when handling any laboratory chemical: safety glasses or goggles, closed toed shoes, laboratory coat, and gloves. Wash hands before and after the use of disposable gloves. Respirators and heat resistant or other specialized hand protection use may be necessary for some heating protocols and other chemical manipulations. Keep workspace clear of unnecessary materials. Label all laboratory chemicals with appropriate hazard signage.
Laceration		Lubricate rubber stoppers before trying to force onto glass. Use gentle pressure with rotation on the glass part. Wear specialized cut resistant hand protection if available. Wash hands before and after the use of disposable gloves. Keep workspace clear of unnecessary materials.
Absorption of Chemicals		Keep chemicals off the skin. Organic substances are absorbed through the skin even if they do not burn or are corrosive. Repeated exposure may result in contact dermatitis. Always wear properly fitted and inspected TRU laboratory mandated personal protective equipment when handling any laboratory chemical: safety glasses or goggles, closed toed shoes, laboratory coat, and gloves. Wash hands before and after the use of disposable gloves. Respirators and heat resistant or other specialized hand protection use may be necessary for some heating protocols and other chemical manipulations. When appropriate, to prevent splash hazards, do as much chemical manipulation as possible so in a certified, operating fume hood with the sash pulled down to a protective level. Keep workspace clear of unnecessary materials. Label all laboratory chemicals with appropriate hazard signage.

Ingestion of Chemicals		Do not ingest any laboratory chemicals. Never use mouth suction for pipettes. Wash hands before and after performing any activities in a TRU laboratory facility. Do not eat or drink in the lab. Do not store food or drink in the lab. To further protect, always wear properly fitted and inspected TRU laboratory mandated personal protective equipment when handling any laboratory chemical: safety glasses or goggles, closed toed shoes, laboratory coat, and gloves. Wash hands before and after the use of disposable gloves. When appropriate, to prevent splash hazards, do as much chemical manipulation as possible so in a certified, operating fume hood with the sash pulled down to a protective level. Keep workspace clear of unnecessary materials. Label all laboratory chemicals with appropriate hazard signage.
Inhalation of Chemicals		NEVER *sniff* a product to establish what it is. If odor of a chemical is necessary, instead waft the chemical. Proper wafting technique includes: First, hold the chemical vessel at a comfortable distance, away from the face near waist level. Next, cup the free hand over the mouth of the vessel and gently move the cupped hand towards the face. Breathe in only as much as required to detect the odor of the chemical in question. Many common solvents are toxic if inhaled in any quantity or over a period of time. When manipulating any laboratory chemicals, when possible do so in a certified, operating fume hood with the sash pulled down to a protective level. Always wear properly fitted and inspected TRU laboratory mandated personal protective equipment when handling any laboratory chemical: safety glasses or goggles, closed toed shoes, laboratory coat, and gloves. Wash hands before and after the use of disposable gloves. Properly fitted respirators use will also minimize chemical inhalation risk. Keep workspace clear of unnecessary materials. Label all laboratory chemicals with appropriate hazard signage.
Biohazardous Infectious Materials		Wash hands with disinfectant soap upon arrival at the lab and before you leave. Food, drink, chewing gum, or smoking/vaping of any kind is strictly prohibited in the microbiology labs. Always wear properly fitted and inspected TRU laboratory mandated personal protective equipment when handling any biological material: safety glasses or goggles, closed toed shoes, laboratory coat, and gloves. Wash hands before and after the use of disposable gloves.

		Always strictly adhere to good aseptic technique when performing microbiological laboratory activities. Disinfect work stations before initiation of any activities. Sterilize all equipment before and after work activities. Keep workspace clear of unnecessary materials. Inoculating loops and needles should be flame sterilized before being laid down on any surface. Always use new, disposable gloves to handle biological material and replace gloves before assuming any unrelated tasks. Inoculating loops and needles should be flame sterilized before being laid down on any surface. Observe Containment Level laboratory practices for the biological materials in use. If Containment Level 1, use good microbiological lab practices. For Containment Level 2 materials, the use of an appropriate Biosafety Cabinet is necessary. Avoid opening biological cultures in the laboratory, unless within a certified, functioning, and disinfected Biosafety Cabinet. Even within these, leaving any vessel or culture should be avoided t avoid culture contamination. Treat all animal and human fluids, cells, cell lines, tissues, organs, blood, and blood fractions as infectious and handle only under Containment Level 2 conditions. Do not recap needles and dispose of used needles, used blades, and broken glass in appropriate sharps containers. Properly dispose of biohazardous waste in accordance with TRU policies. If centrifuging biological materials, ensure vessel caps are tightly closed and the centrifuge is well balanced to prevent the generation of aerosols. Label all laboratory chemicals with appropriate hazard signage.
EYE INJURY Chemical splashes		Chemicals may splash into eyes during pouring or use – wear chemical splash goggles and full-face shield. When appropriate, to prevent splash hazards, do as much chemical manipulation as possible so in a certified, operating fume hood with the sash pulled down to a protective level.

4. ASSOCIATED DOCUMENTATION

Doc. Number	Doc. Title
	Lab Risk Assessment and Control Form
	Training Records
	Incident Investigation Form

5. PROCEDURES AND RESPONSIBILITIES

5.1. Containment Level 1 Laboratory Practices:

5.1.1. Laboratory doors should be kept closed and access to laboratory areas must be limited and controlled.

5.1.2. People must be advised of potential hazards before entering the work area.

5.1.3. Mouth pipetting is strictly prohibited.

5.1.4. Eating, drinking, smoking, vaping, applying cosmetics, handling contact lenses, chewing gum and/or storing food is not permitted in the laboratory areas.

5.1.5. Work surfaces should be decontaminated before and after work activities and after any spill.

5.1.6. Work areas should be clear of clutter.

5.1.7. Employees must wash their hands upon arrival at the laboratory, before and after putting on protective disposable gloves, after handling infectious materials, and before leaving the laboratory.

5.1.8. All spills, accidents and possible exposures to infectious materials must be reported immediately to the Laboratory Supervisor and the University Biosafety Officer.

5.1.9. The Lab Supervisor will ensure that training in laboratory safety for infectious materials is provided. This includes but is not limited to:

a) Technical training, exposure prevention precautions, and exposure evaluation procedures

b) Informational and technical updates and additional training when required.

c) Personal health precautionary materials, which can include relevant vaccine information, specialized risk precautions for pregnant or immunocompromised individuals

d) If a member of an increased risk group, encourage self-identification to campus Health Services for appropriate counseling and guidance.

5.1.10. All contaminated or infectious liquid or solid materials must be decontaminated before disposal or re-use.

5.1.11. Where infectious agents are used in a laboratory, a biohazard warning sign incorporating the universal biohazard symbol must be posted on the access door to the work area.

5.1.12. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. If transporting waste prior to decontamination, ensure materials are placed in a durable, leak-proof container and packed in accordance with applicable institutional, local, provincial, and federal regulations.

5.1.13. Safe handling of sharps includes:

a) Disposable needles are not to be manipulated, bent, sheared, broken, removed from syringe base or recapped before disposal. Likewise, blades, broken glass or other sharps must also be treated with care and not handled or manipulated prior to disposal.
b) Used disposable needles and syringes must be carefully placed in a convenient, puncture resistant sharps container.
c) Non-disposable sharps are to be placed in a convenient hard walled container for transport to a decontaminating area.
d) Broken glass is not to be handled directly. Alternatively, all broken glass is to be collected with a broom and dustpan, tongs, or forceps. Substitute plastic for glass whenever possible.

5.1.14. An applied effective laboratory pest management program is required for safe laboratory operations.

5.1.15. Equipment must be decontaminated before removal from laboratory for service and/or repair.

5.1.16. Laboratory furniture must be in good repair.

5.2 Inoculation of Culture Media

5.2.1 For microbiological investigations it is essential to learn the skills of inoculating specimens onto culture media:

5.2.2 Always practice aseptic technique clean work area with supplied disinfectant before beginning your work and upon completion.

5.2.3 Ensure loops and picks are flamed upon completion of your work

5.2.4 Discard any waste bio-hazardous material in the appropriate area, to ensure adequate disinfection is completed.

5.3 Containment Level 1 Laboratory Personal Protective Equipment Practices.

5.3.1 At a minimum, a lab coat, closed-toe shoes, eye protection (when necessary), and protective, disposable gloves must be worn in any microbiology laboratory. This equipment prevents bio-hazardous materials from contact with the skin and eyes, including areas where there might be cuts, abrasions, or dermatitis.

5.3.2 Prior to initiating any work, review the relevant SDS and PSDS associated with the intended activity and keep them close at hand for quick reference.

5.4 Lab Coats:

5.4.1 Must be worn by all personnel, including visitors, trainees and others entering or working in the laboratory.

5.4.2 Coats must be properly fastened.

5.4.3 Prior to donning a lab coat, carefully inspect the lab coat for holes, tears, evidence of contamination, and inside the pockets for debris. If any of these are found, notify lab supervisor/staff and obtain a new lab coat, which must be inspected for holes, tears, contamination, and debris prior to use. Only use lab coats that are free from the above deficiencies.

5.4.4 If contaminated, lab coats should be decontaminated by autoclaving before being placed in the laundry. If decontamination is not possible, any contaminated coat should be placed in the biohazard waste container.

5.4.5 Other articles of clothing, if contaminated during the course of lab work must also be likewise

decontaminated.

5.5 Gloves:

5.5.1 Must be worn for all procedures performed in the microbiology laboratories involving infectious or potentially infectious materials.

5.5.2 Glove selection should be based on appropriate risk assessment (Table 1).

5.5.3 Latex or nitrile gloves offer a high level of dexterity and a higher level of sensitivity; however, they do not offer a great deal of protection from needle sticks, animal bites or sharps.

5.5.4 Some procedures may require double gloving.

5.5.5 Prior to donning gloves, inspect them for thinning areas, holes, tears, and other imperfections that could impede their protective qualities. Discard gloves with any of the above imperfections and obtain new gloves. Only use gloves that are free from deficiencies that could impede their protective functions.

5.5.6 Change gloves periodically during work functions and also when they become contaminated, their integrity is compromised, or when otherwise necessary.

5.5.7 Do not wash or reuse disposable gloves.

5.5.8 Gloves must be removed prior to leaving the laboratory and placed in a biohazard waste receptacle for decontamination with other laboratory wastes before disposal.

5.5.9 Safe glove removal includes: grasping one glove at the top of your wrist, being careful not to touch bare skin. Peel this glove off, away from your body, turning it inside out. Hold that glove you just removed in your gloved hand. Insert non-gloved hand into the cuff of the glove at the top of your wrist. Turn this glove inside out while tilting it away from your body. Dispose of the gloves – do not reuse.

Table 1: Available glove types and their respective advantages and disadvantages.

TYPE	ADVANTAGES	DISADVANTAGES	FOR USE WITH:
Natural rubber latex and rubber blends	**Good Biological Protection.** Low cost, good physical properties, dexterity.	Poor for solvent use and ethidium bromide. May cause allergic reactions.	**Biological Materials.** Aqueous solutions, bases, acids, alcohols, dilute aqueous solutions.
Polyvinyl chloride (PVC)	**Good Biological Protection.** Low cost, very good physical properties, average chemical resistance.	Plasticizers can be stripped.	**Biological Materials.** Strong acids and bases, salts, aqueous solutions, alcohols, oils, greases and petroleum products.
Neoprene	**Good Biological Protection.** Average cost, average chemical resistance, average physical properties, high tensile strength, high heat resistance.	Poor vs. chlorinated hydrocarbons	Oxidizing acids, alcohols, anilines, phenol, glycol ethers, solvents, oils, mild corrosives
Nitrile	**Excellent Biological Protection.**	Poor vs. chlorinated organic solvents	**Biological Materials. Syringe/Needle work.**

	Best needle wiping capacity. Low cost, excellent physical properties, dexterity.		Oils, greases, aliphatic hydrocarbons, xylene, perchloroethylene, trichloroethane, ethidium bromide. Fair vs. toluene.
Butyl	Good resistance to polar organics, high resistance to gas and water vapour	Expensive, poor vs. hydrocarbons, chlorinated solvents	Glycol ethers, ketones, esters, aldehydes, polar organic solvents
Polyvinyl alcohol (PVA)	Resists broad range of organics, good physical properties.	Very expensive. Water sensitive, poor vs. light alcohols, acids and bases.	Aliphatic and aromatic hydrocarbons, chlorinated solvents, ketones (except acetone), esters, ethers
Fluro-elastomer (Vitron®)	Good resistance to organic and aromatic solvents. Flexible.	Extremely expensive. Poor physical properties. Poor vs. some ketones, esters, amines	Aromatics and aliphatic hydrocarbons, chlorinated solvents, oils, lubricants, mineral acids, alcohols.
Norfoil, Silver Shield™, 4H™	Excellent chemical resistance.	Poor fit, stiff, easily punctures, poor grip.	Use for Hazmat work. Good for range of solvents, acids and bases.

5.6 Protective Eyewear:

5.6.1 The use of contact lenses in laboratories is discouraged. Instead wear safety glasses on top of prescription lensed glasses for work functions, or alternatively, use prescription safety glasses.

5.6.2 Protective eyewear must be worn when aerosols and splashes are a risk or when large volumes are being used.

5.6.3 Protective eyewear should fit comfortably and snugly while not interfering with personnel activities.

5.6.4 Safety glasses are sufficient protection for most laboratory activities.

5.6.5 If there is increased splash risk personnel should instead wear protective goggles or a full face shield.

5.6.6 Damaged eye protection should be replaced immediately.

5.7 Closed toed shoes:

5.7.1 Closed toed shoes are mandatory laboratory PPE.

5.7.2 Sandals and other similar open toed shoes are forbidden in laboratory areas.

5.7.3 Closed toed shoes should be non-slip and provide full foot protection.

5.7.4 Safety laboratory shoes are not required but provide additional protection from chemical, biological, thermal, electrical, and kinetic hazards.

5.8 Containment Level 2 Laboratory Practices:

5.8.1 All containment level 1 practices detailed in previous sections are to be applied, however other precautions that are necessary follow below.

5.8.2 Personnel should be provided with appropriate medical surveillance and offered immunizations for infectious agents they may be exposed to during work activities.

5.8.3 Consideration for collection and storage of serum samples should be carried out for at-risk personnel.

5.8.4 Biosafety manual and approved certificate describing permissible laboratory activities must be made available to relevant personnel.

5.8.5 Adequate training for laboratory staff in standard and specialized microbiological techniques by the Laboratory Supervisor prior to working with BSL-2 materials.

5.8.6 Demonstrated competency by laboratory staff in regular and specialized microbiological techniques by the Laboratory Supervisor prior to working with BSL-2 materials.

5.8.7 Potentially infectious exposure incidents must be evaluated immediately and treated according to procedures described in the laboratory Biosafety Certificate. All such incidents must be recorded and reported to and addressed by the laboratory supervisor, the TRU Biosafety office, and health services.

5.8.8 Animals and plants not associated with the projects that are being conducted must not be permitted in the laboratory.

5.8.9 All procedures involving the manipulation of biological material that could generate an aerosol should be conducted within an operational, certified Biosafety Cabinet (BSC).

5.9 Additional Personal Protection, Safety Practices, and Equipment for Containment Level 2 Laboratory Operation:

5.9.1 All containment level 1 practices detailed in previous sections are to be applied, however other precautions that are necessary follow below.

5.9.2 Properly maintained and certified BSCs, other appropriate PPE, and other physical containment devices must be used during:

5.9.3 All work with human blood, blood fractions, cells, cell lines, tissues, and organs where there is splash or aerosol generation risk.

5.9.4 Procedures and activities where splash or aerosol generation could occur. This can include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers, opening infectious materials, intranasally inoculating animals, and harvesting infected tissues from animals or eggs.

5.9.5 All procedures which involve high concentrations and/or large volumes of infectious materials and/or toxins.

5.9.6 Additional protective gowns, aprons, coats, coveralls, smocks, or scrubs should be worn when available to prevent personnel contact with infectious agents or biological materials.

5.9.7 Contaminated protective clothing should be disposed of in the hazardous waste receptacles or laundered appropriately via institutional guidelines.

5.9.8 BSCs must be installed so that fluctuations in air supply and exhaust do not interfere with normal operations. They should also be away from doors, windows that open, heavy traffic

areas, and other sources of possible airflow disruption.

5.9.9 BSC vacuum lines should be protected with disinfectant traps.

5.9.10 An eye wash station should be readily available.

5.9.11 Only HEPA filtered exhaust air from a certified and tested Class II BSC, can be safely recirculated back into the laboratory environment.

5.9.12 Lab doors must lock and laboratory furniture must be in good repair.

5.10 Inoculation of Culture Media within a BSC for CL2

5.10.1 Containment level 1 practices (section 5.2) will be observed however, additional care is required for safe work in a BSC.

5.10.2 Cabinet blowers should be engaged at least 5 minutes before initiating work in a BSC. Never operate BSC blower wish sash lowered completely.

5.10.3 Prior to engaging in work within the BSC, the interior surfaces of the BSC should be decontaminated with 70% isopropyl or ethyl alcohol or other specified decontaminant, where effective for target organisms.

5.10.4 Decontaminant choice and contact times depend on the biological material in question and therefore, different decontamination protocols and materials may be required for safe BSC operation and decontamination. For a general guide to BSC decontamination with various biological materials, see Table 2.

5.10.5 According to the 2016 Canadian Biosafety Standard, published by PHAC, moist heat (autoclaving in excess of 121°C for 60 minutes) will permit adequate inactivation of most biological toxins. However, this is not suitable for inactivation of low-weight, heat-stable toxins (e.g. Anthrax). Similarly, 30 minutes of contact with a solution of 2.5% NaOCl and 0.25N NaOH is adequate for inactivation of most biological toxins.

5.10.6 In some cases, however, different protocols are required. Please see Table 3 in this document for some examples of effective biological toxin decontaminating agents. If the toxin in use does not appear on this list, please contact the Biosafety Office for decontamination protocols.

5.10.7 If unsure of anything to do with toxins, please contact the Biosafety Office – NEVER work with a biological toxin with which you are unsure of PPE or decontamination requirements and protocols.

5.10.8 If a 10% bleach solution is to be used to decontaminate the interior of the cabinet, its application should be followed by removal with abundant sterile water or 70% isopropyl or ethyl alcohol.

5.10.9 Materials that are to be placed inside the cabinet should be surface-decontaminated with 70% isopropyl or ethyl alcohol.

5.10.10 No paper or other objects should be allowed to obstruct the front grill of the BSC.

5.10.11 Do not use an open flame in a BSC.

5.10.12 Personnel should plan their work ahead such that sweeping arm movements within the cabinet are limited. This can be solved by dividing the interior of the cabinet into clean and

dirty working areas.

5.10.13 While working, keep sash lowered enough to maintain BSC interior isolation, but also so that personnel can work comfortably.

5.10.14 Any materials that are removed from inside the cabinet should be surface-decontaminated with 70% isopropyl alcohol, unless otherwise specified.

5.10.15 Discard any waste bio-hazardous material in the appropriate area, to ensure adequate disinfection is completed.

5.10.16 Once work is completed, decontaminate the interior of the BSC as previous described, turn off the blower and completely lower the sash.

5.10.17 If UV lamps are used as an infection prevention device, they must be regularly cleaned and tested every 6 months to ensure adequate energy output. The sash must also be completely lowered if UV lighting is engaged.

5.10.18 The radiation output of the lamp must be measured routinely (at least twice yearly) with a UV meter to ensure that the proper intensity (40 µW/cm2) and wavelength (254 nm) are being delivered to the work area.

Table 2: Disinfectant Selection for various pathogens.

Biological Material	Chlorine Compounds (10% household bleach, make fresh monthly)	Alcohols (70% solutions most effective)	Phenolics (dilute according to manufacturer's instructions – useful for organic matter clean up)	Quaternary Ammonium Compounds (cationic detergents)	Glutaraldehyde	Formaldehyde
Bacteria	Very good	Good	Good	Good for gram positive	Good	Good
Enveloped viruses	Very good	Good	Good	Good	Good	Good
Non-enveloped viruses	Very good	Virus-dependent	Virus-dependent	Ineffective	Fair, 20 min contact time	Good
Fungi	Good	Fair	Good	Fair	Good	Good
Bacterial spores	Good with high concentration	Ineffective	Ineffective	Ineffective	Fair, 30 min contact time	Good
Protozoal parasites	Moderate with high concentration and several hours of treatment	Ineffective	Ineffective	Fair at high concentrations	Good	Good
Prions	**Special** – require 1M of this or NaOH for 60 minutes and then autoclave for 1 hour at 121°C – preferable to use disposable instruments and lab ware	Ineffective	Ineffective	Ineffective	Ineffective	Good

Table 3: Example Toxin Inactivation Guide

Toxin	Autoclave, 60min, 121°C	NaOCl, 30 min	NaOCl + NaOH, 30 min	Remarks
Abrin	YES	0.1%	Not determined	
Anthrax Lethal Toxin	YES	0.5%	2.5% NaOCl +0.25 NaOH	
Botulinum neurotoxins	YES	0.1%	2.5% NaOCl +0.25 NaOH	
Brevetoxin	NO	2.5%	2.5% NaOCl +0.25 NaOH	
Cholera toxin	YES	0.5%	Not determined	
Conotoxin	NO	0.5%	Not determined	30 minute treatments of 1% (v/v) solutions of glutaraldehyde or formaldehyde are also effective.
Deoxinalenol	NO	2.5%	2.5% NaOCl +0.25 NaOH	
Diacetoxyscirpenol	NO	NO	2.5% NaOCl +0.25 NaOH	
Diphtheria toxin	YES	0.5%	2.5% NaOCl +0.25 NaOH	
Microcystin	NO	0.5%	2.5% NaOCl +0.25 NaOH	
Ricin		0.1%	2.5% NaOCl +0.25 NaOH	
Saxitoxin	NO	0.1%	2.5% NaOCl +0.25 NaOH	

Shigatoxin and Shiga-like ribosome inactivating proteins	YES	0.1%	2.5% NaOCl +0.25 NaOH	
Staphylococcal enterotoxins	YES	0.5-1.0%	Not determined	60 minute contact time required
T-2 mycotoxin	NO	NO	***2.5% NaOCl +0.25 NaOH	2-8 hour soak required for gross contamination
Tetanus toxin	YES	0.5%	Not determined	
Tetrodotoxin	YES	1.0%	2.5% NaOCl +0.25 NaOH	

5.11 Spills in a Biological Safety Cabinet

5.11.1 Spill Clean Up in a BSC:

a) Assess situation and personal contamination. Ensure risk of injury is controlled prior to initiating spill cleanup.

b) Remove gloves and discard within the BSC.

c) If two pairs of gloves are being worn, discard only outermost layer.

d) Remove any other contaminated and potentially contaminated PPE and clothing.

e) If sleeves are contaminated, the lab coat or gown should also be removed.

f) Leave BSC blower on and do not move sash.

g) Notify all staff in the immediate vicinity of the spill and to leave the area for 30 minutes to allow aerosols to settle, using normal exit procedures.

h) Exposed persons should wash any potentially exposed skin areas thoroughly with soap and running water and encourage bleeding if exposure involves a sharps injury or puncture and keep washing.

i) Exposed persons should be referred for immediate medical attention.

j) Inform the laboratory supervisor or responsible authority immediately.

k) Post signage forbidding entry to immediate spill area for the settling period.

l) After 30 minutes has elapsed, don fresh, inspected, risk-appropriate PPE (double glove, closed toed shoes, laboratory coat, and eye protection minimum). LRA will determine if more is needed (e.g. a respirator).

m) If the spill involves body fluids, blood, or human cells, ensure adequate skin coverage.

n) Assemble biological spill kit and bring it to the spill site.

o) Gently cover the spill with paper towel or cloth to contain it.

p) Gently pour an appropriate disinfectant on the paper towel or cloth, starting at the outer margin of the spill areas and working concentrically towards the center of the spill.

q) Allow for appropriate contact time.

r) Replace any PPE that was contaminated by the initial decontamination steps.

s) After contact time has elapsed, carefully remove the towels/cloth and any debris. If there is broken glass remove using a dustpan and broom, forceps etc.

t) Clean and disinfect the area. Dispose of the contaminated materials in a leak-proof, puncture resistant waste disposal container. Repeat if necessary.

u) Remove contaminated PPE and don clean PPE.

v) After disinfection notify the laboratory supervisor and the BSO that the site has been decontaminated.

w) Allow BSC to purge for at least 10 minutes before resuming work or shut down.

x) If the spill has breached through the front or back grills, those will have to be cleaned as well (see instructions on catch basin cleaning below).

5.11.2 Catchbasin Decontamination:

a) Find a partner to do this.

b) Identify drain valve.

c) Close drain port.

d) Presoak paper towels in an effective decontaminant.

e) Place a bucket under the drain and attach tubing long enough to reach from the drain to the inside of the bucket.

f) Wet top surface of front grille 3 times with pre-soaked paper towels.

g) Remove the grille and place it on the BSC work surface.

h) Wet wipe remainder of internal surfaces.

i) Spray front grille with 70% alcohol, allow 5 minutes for contact time.

j) Remove grille from BSC and place in a sink.

k) WITH A PARTNER, lift and prop up the work space securely.

l) If necessary, clean the underside of the work surface with wet paper towels.

m) Rinse with paper towels soaked in 70% alcohol or sterile water.

n) Using a flashlight and an extendable mirror (if available), identify “how much” cleaning will have to get done.

o) Saturate the inside of the catch basin with a misting with a decontaminating agent. Do not let this dry, so keep misting if need be.

p) Loosen moistened debris with a plastic scraper or other similar implement to avoid

damaging the BSC.

q) Carefully collect debris (if sharps, use forceps).

r) Flood catchbasin with no more liquid than the bucket can hold, then scrub the inside of the catch basin with a brush of some kind (eg. toilet).

s) Let sit 15 for recommended decontaminant contact time.

t) If unable to drain, absorb the decontaminant with paper towels, otherwise drain liquid into bucket.

u) Repeat steps o – t with decontaminant and then with tap water to remove corrosive chemicals.

v) Mist catch basin with 70% alcohol and allow to dry.

w) Replace work surface and front grille and decontaminate both.

5.11.3 Small spill (RG1 material spill without splashing or agitation **or** <100mL volume and low concentration of RG2) outside of BSC:

a) Assess the situation and personal contamination. Ensure risk of injury is controlled prior to initiating spill cleanup.

b) Remove any contaminated and potentially contaminated PPE and clothing.

c) If sleeves are contaminated, lab coat or gown should be removed and placed in an autoclave bag for decontamination.

d) Notify all staff in the immediate vicinity of the spill and have everyone leave the area for 30 minutes to allow for aerosol settling, using normal exit procedures.

e) Exposed personnel should wash any potentially exposed skin areas thoroughly with soap and running water.

f) Encourage bleeding if exposure involves a sharps injury or puncture and keep washing.

g) Exposed persons should be referred to immediate medical attention.

h) Inform the laboratory supervisor or responsible authority immediately.

i) Post a sign forbidding entry to immediate spill area for the settling period.

j) After 30 minutes has elapsed, don fresh, inspected, risk-appropriate PPE (double glove, closed toed shoes, laboratory coat, and eye protection minimum). LRA will determine if more is needed (e.g. a respirator).

k) If the spill involves body fluids, blood, or human cells, ensure adequate skin coverage and wear a face mask in addition to the above listed PPE.

l) Assemble the biological spill kit and bring it to the spill site.

m) Gently cover the spill with paper towel or cloth to contain it.

n) Gently pour an appropriate disinfectant on the paper towel or cloth, starting at the outer margin of the spill areas and working concentrically towards the center of the spill.

o) Allow for appropriate contact time.

p) Replace any PPE that was contaminated during initial clean up steps.

q) After contact time has elapsed, carefully remove the towels/cloth and any debris. If there is broken glass, remove this using a dustpan and broom, forceps etc.

r) Clean and disinfect the area and dispose of the contaminated materials in a leak-proof, puncture resistant waste disposal container. Repeat if necessary.

s) Remove contaminated PPE and don clean PPE.

t) After disinfection notify the laboratory supervisor and the BSO that the site has been decontaminated.

5.11.4 Large Spill (**if NON-biological**):

a) Do not panic. Stay calm.

b) Remove any contaminated and potentially contaminated PPE and clothing.

c) Notify all staff in the immediate vicinity of the spill and have everyone leave the area.

d) Pull fire alarm and evacuate everyone.

e) Avoid touching or attempting to clean up the spill.

f) Contact security at 1111 or 9-828-5033 from a safe location and provide the following details:

i. Spill location

ii. Nature of the hazardous material/biohazardous material

iii. Quantity involved

iv. Related health hazards and precautions to be taken

g) Attempt to administer first aid if necessary and if properly trained. However, do not contaminate yourself or others.

h) Do what you can to prevent others from entering the spill area.

i) Ensure your own personal safety.

j) Notify other staff and evacuate the area and await further instructions from trained personnel.

5.11.5 Large Spill (**if biological**):

a) Assess incident severity and personal contamination – remember personal exposure takes priority over clean up.

b) Ensure risk of injury is controlled prior to initiating spill cleanup.

c) Remove any contaminated and potentially contaminated PPE and clothing.

d) If sleeves are contaminated, lab coat or gown should be removed and placed in an autoclave bag for decontamination. Optimally this would be done in proximity to the spill.

e) Notify all staff in the immediate vicinity of the spill and have everyone leave the area for 30 minutes to allow for aerosol settling, using normal exit procedures.

f) Exposed personnel should wash any potentially exposed skin areas thoroughly with soap and running water.

g) Encourage bleeding if exposure involves a sharps injury or puncture and keep washing.

h) Exposed persons should be referred for immediate medical attention.

i) Inform the laboratory supervisor or responsible authority immediately.

j) Post a sign forbidding entry to immediate spill area for the settling period.

k) Ask yourself if you have the experience and ability to clean up the spill – if not, notify your supervisor or senior lab staff who will instruct in the cleaning protocol.

l) After 30 minutes has elapsed, don fresh, inspected, risk-appropriate PPE (double glove, closed toed shoes, laboratory coat, and eye protection minimum). LRA will determine if more is needed (e.g. a respirator).

m) If the spill involves body fluids, blood, or human cells, ensure adequate skin coverage and wear a face mask in addition to the above listed PPE.

n) For respiratory transmitted biological materials, the PI should determine if an N100 or HEPA filtered respirator is necessary.

o) Assemble the biological spill kit and bring it to the spill site.

p) Gently cover the spill with paper towel or cloth to contain it.

q) Gently pour an appropriate disinfectant on the paper towel or cloth, starting at the outer margin of the spill areas and working concentrically towards the center of the spill.

r) Allow for appropriate contact time.

s) Replace any PPE that was contaminated during initial clean up steps.

t) After contact time has elapsed, carefully remove the towels/cloth and any debris. If there is broken glass, remove this using a dustpan and broom, forceps etc.

u) Clean and disinfect the area and dispose of the contaminated materials in a leak-proof, puncture resistant waste disposal container. Repeat if necessary.

v) Remove contaminated PPE and don clean PPE.

w) After disinfection notify the laboratory supervisor and the BSO that the site has been decontaminated.

x) Depending on the size and/or nature of the spill, a complete room decontamination may be warranted – this will be determined by the PI and the BSO.

6.0 EQUIPMENT

6.1 CENTRIFUGES

6.1.1 Safe use of centrifuges requires proper maintenance and operation. Failed mechanical parts or improper operation can result in release of projectiles, hazardous chemicals and bio-hazardous aerosols. Maintenance and repairs must be performed only by trained, qualified personnel. To maintain your safety, sample integrity and the equipment, follow these guidelines.

6.1.2 Ensure that centrifuges have an interlocking device that will prevent both the lid from being opened when the rotor is in motion and the centrifuge from starting when the lid is open.

6.1.3 Ensure that centrifuge tubes are free of hairline cracks, stress lines and chipped rims prior to use.

6.1.4 Ensure that tube materials are chosen such that they provide the necessary chemical resistance and speed rating.

6.1.5 Avoid over-filling tubes.

6.1.6 Cap or stopper centrifuge tubes.

6.1.7 Use sealed centrifuge buckets (safety cups) or rotors that can be loaded and unloaded in a biological safety cabinet or chemical fume hood as appropriate.

6.1.8 Decontaminate the outside of the cups/buckets and rotors before and after centrifugation.

6.1.9 Inspect O-rings regularly and replace if they are cracked or dry.

6.1.10 Ensure that the centrifuge is properly balanced. Load the rotor with samples arranged symmetrically. Opposing tubes must be of equal weight. If necessary, use "water blank" tubes to balance sample tubes of unequal weigh. Do not use sight or volume to conclude that tubes are balanced. Use an electronic balance to balance tube before using them in an ultracentrifuge.

6.1.11 Ensure that the prescribed speed limitations of the rotor or centrifuge are never exceeded.

6.1.12 Unless fitted with a suitable exhaust system, do not centrifuge materials capable of creating flammable or explosive vapours.

6.1.13 Remain with the centrifuge until it has reached its programmed speed.

6.1.14 Abort the run immediately if you hear abnormal vibration, whining or grinding noises. Check the rotor lid and balance.

6.1.15 At the end of the run ensure that the rotor and centrifuge are cleaned according to manufacturer’s instructions. Never use abrasive cleaners.

6.1.16 Rotors are easily damaged. Never use metal tools to remove tubes or clean the rotors.

6.1.17 If the centrifuge is connected to a vacuum pump, ensure that the pump exhaust is connected to a trap.

6.1.18 If bio-hazardous materials are being centrifuged and the centrifuge is connected to a vacuum pump, ensure that a HEPA filter is installed between the centrifuge and the vacuum pump.

6.1.19 In the event of a spill, follow instructions in previous sections.

6.2 BLENDERS, GRINDERS AND SONICATORS

6.2.1 When used with infectious agents, mixing equipment such as shakers, blenders, grinders, sonicators and homogenizers can release significant amounts of hazardous aerosols, and should be operated inside a biological safety cabinet whenever possible.

6.2.2 Ensure equipment has safety features that will minimize leaking and prevent operation if

blades are exposed.

6.2.3 Ensure that any equipment that could move during use is secured to a bench or the floor as applicable.

6.2.4 Ensure equipment is in good condition prior to use.

6.2.5 Allow aerosols to settle for at least one minute before opening containers.

6.2.6 Do not use flammable solvents in equipment such as blenders and stirrers as they can also produce a large amount of flammable vapours.

6.2.7 In the event of a spill, follow instructions in section 6.9.

6.3 ELECTROPHORESIS

6.3.1 The use of voltages of approximately 200 V and currents of more than 80 mA in electrophoresis procedures could create the potential for an electrical shock if the equipment is not operated properly.

6.3.2 Use physical barriers to prevent inadvertent contact with the equipment.

6.3.3 Ensure that electrophoresis equipment is properly grounded.

6.3.4 Inspect electrophoresis equipment regularly for damage and potential buffer tank leaks.

6.3.5 Locate equipment away from high traffic areas and away from wet areas such as sinks or washing apparatus.

6.3.6 Use of ground fault circuit interrupters is recommended.

6.3.7 Display warning signs to identify the electrical hazards (i.e. “Danger – High Voltage”).

6.3.8 Turn off power before connecting leads, opening the lid or reaching into the chamber.

6.3.9 Ensure that lead connectors are insulated.

6.4 GAS CHROMATOGRAPHS

6.4.1 Gas chromatography (GC) procedures involve the use of compressed gas cylinders and may involve the use of flammable solvents and toxic chemicals. Be familiar with the use and handling of compressed gas cylinders, and with hazardous properties, precautionary measures, and handling instructions for any hazardous materials being used. Refer to MSDSs (found on-line using the MSDSonline icon on the desk top) or other reliable reference material. The following guidelines will assist in the safe operation of GCs.

6.4.2 Wear proper eye protection. GC columns are fragile and breakage could result in small projectiles during handling. As well, samples are prepared in various hazardous solvents that could damage the eyes upon contact.

6.4.3 When cutting a GC column, be sure that the cut is made away from the body.

6.4.4 Ensure that GC column cutters are capped or otherwise stored to prevent injury when not in use.

6.4.5 Discard small pieces of GC columns as sharps waste.

6.4.6 Ensure that the oven is allowed to cool before installing or removing a column or injector or prior to performing maintenance.

6.4.7 Ensure that gases are turned off prior to removing or installing a column.

6.4.8 Test for leaks after the installation of the column and whenever a leak is suspected. Use a technique that will not damage or sacrifice the integrity of the instrument.

6.4.9 Electron capture detectors (ECD) have a radioactive source and therefore need to be registered as part of the University's Radiation Safety program. ECDs may not be relocated or discarded without permission of the Radiation Safety Officer. Contact the Radiation Safety Officer at extension 2874 for more information about Canadian Nuclear Safety Commission (CNSC) requirements.

6.4.10 Ensure that the instrument and gases are turned off and the power cord disconnected prior to performing maintenance.

6.5 GLASSWARE

6.5.1 Improper use of glassware can lead to injuries in the laboratory. The following guidelines will help in the safe use of glassware.

6.5.2 Use only the right size and type of glassware for any given operation.

6.5.3 Ensure that glassware is in good condition prior to use (i.e. no cracks, chips, significant scratches).

6.5.4 Discard broken glassware in appropriate containers.

6.5.5 Cut glass tubes/tubing by scoring using a file or equivalent. Cover the glass with a piece of cloth and break at the score over a piece of cloth/paper to catch any pieces.

6.5.6 Wear leather or other cut-resistant gloves when inserting glass tubing into a stopper or flexible tubing. Fire polish tubing ends and lubricate glass to make the connection easier. Ensure that stopper holes are appropriately sized and carefully insert tubing by carefully twisting back and forth.

6.5.7 Wear leather gloves when removing glass tubing from flexible tubing or a stopper. If difficult, carefully cut with a scalpel blade or other appropriate glass cutter. Ensure that cuts are made away from the body.

6.5.8 Ensure glassware is stored away from the edges of benches so that it cannot easily be knocked.

6.6 HEATING BATHS

6.6.1 Heating baths are designed to heat materials to constant temperature. They may be filled with a variety of materials including water, mineral oil, sand, glycerin, paraffin or silicone oils, depending on the bath temperature required. The following are precautions for heating baths:

6.6.2 6.6.2 Locate on a stable surface, away from flammable and combustible materials including wood and paper.

6.6.3 Ensure liquid has cooled before moving the heating bath,

6.6.4 Do not over fill the bath.

6.6.5 Ensure baths are equipped with controls that will turn off the power if the temperature exceeds a preset limit.

6.6.6 Ensure that the thermostat is set well below the flash point of the heating liquid in use.

6.6.7 Equip the bath with a non-mercury thermometer to allow a visual check of the bath temperature.

6.6.8 Take care not to allow water to get into oil baths as violent splattering may result.

6.6.9 Steams baths are often safe alternatives for heating because they provide a consistent temperature that will not exceed 100°C. However, care must be taken to prevent scalding due to dermal exposure to the steam or steam lines.

6.6.10 Water baths are the most common heating bath found in the laboratory. When using a water bath:

6.6.11 Clean the bath regularly; a disinfectant can be added to the water,

6.6.12 Decontamination can be performed by raising the temperature to 90°C or higher for 30 minutes once a week.

6.6.13 Unplug the unit before filling or emptying.

6.7 HIGH PERFORMANCE LIQUID CHROMATOGRAPHS

6.7.1 High performance liquid chromatography (HPLC) procedures often require handling of flammable and toxic solvents. Refer to MSDSs (found on-line using the MSDS online icon on the desk top) or other reliable reference material. The following guidelines will assist in the safe operation of HPLCs.

6.7.2 Wear appropriate eye protection. Since the HPLC is operated at high pressures, it is possible for fittings to fail, resulting in a sudden release of solvent.

6.7.3 Use “elephant trunk” ventilating system above fraction collectors, especially with normal phase HPLC.

6.7.4 Inspect and empty the waste containers as required.

6.7.5 Ensure that waste collection vessels are vented.

6.7.6 Ensure secondary containment of waste containers.

6.7.7 Never clean a flow cell by forcing solvents through a syringe: syringes under pressure can leak or rupture, resulting in sudden release of syringe contents.

6.7.8 High voltage and internal moving parts are present in the pump and auto sampler. Switch off the electrical power and disconnect the power cord when performing routine maintenance.

6.8 OVENS, HOT PLATES AND HEATING MANTLES

6.8.1 Ovens are commonly used in the lab to evaporate water from samples, provide a stable elevated environment and to dry glassware. Heating mantles are used to heat reaction or sample solutions in round-bottom flasks or reaction vessels, and hot plates are used to heat various general laboratory solutions. Bunsen burners may be used only after obtaining approval from the supervisor. The following precautions should be followed to ensure safe

use.

6.8.2 Ensure that laboratory ovens and hot plates are designed to prevent contact between flammable vapours and heating elements/spark-producing components.

6.8.3 Avoid heating toxic, even mildly volatile materials in an oven unless it is continuously vented outdoors.

6.8.4 Glassware that has been rinsed with an organic solvent is to be rinsed with distilled water or equivalent before being placed in an oven for drying.

6.8.5 Hot plates or ovens whose thermostat fails must be removed from service until repaired. Heating devices whose temperature rises above that required could create significant fire hazards.

6.8.6 Heating mantles must be used in conjunction with a variable autotransformer; care must be taken not to surpass the maximum voltage of the mantle recommended by the manufacturer.

6.8.7 Discontinue use of any heating mantle where the heating elements have become exposed.

6.9 BIOLOGICAL SPILLS IN AND ON EQUIPMENT

6.9.1 Assess situation and personal contamination. Ensure risk of injury is controlled prior to initiating spill cleanup.

6.9.2 Remove any contaminated and potentially contaminated PPE and clothing.

6.9.3 If sleeves are contaminated, lab coat or gown should be removed and placed in an autoclave bag for decontamination.

6.9.4 Notify all staff in the immediate vicinity of the spill and have everyone leave the area for 30 minutes to allow for aerosol settling, using normal exit procedures.

6.9.5 Exposed personnel should wash any potentially exposed skin areas thoroughly with soap and running water.

6.9.6 Exposed persons should be referred to immediate medical attention.

6.9.7 Inform the laboratory supervisor or responsible authority immediately.

6.9.8 Post a sign forbidding entry to immediate spill area for the settling period.

6.9.9 After 30 minutes has elapsed, don fresh, inspected, risk-appropriate PPE (double glove, closed toed shoes, laboratory coat, and eye protection minimum). LRA will determine if more is needed (e.g. a respirator).

6.9.10 If the spill involves body fluids, blood, or human cells, ensure adequate skin coverage and wear a face mask in addition to the above listed PPE.

6.9.11 Consult the operating manual to identify a decontaminating solution that will be both effective against the offending pathogen and that will not compromise aspects of the centrifuge itself.

6.9.12 Assemble the biological spill kit and bring it to the spill site.

6.9.13 Disinfect centrifuge components using paper towel soaked in the selected disinfectant.

6.9.14 Allow for appropriate contact time.

6.9.15 After contact time has elapsed, carefully remove the towels/cloth and if there is broken glass remove using a dustpan and broom, forceps etc.

6.9.16 Clean and disinfect the area and dispose of the contaminated materials in a leak-proof, puncture resistant waste disposal container. Repeat if necessary.

6.9.17 Remove contaminated PPE and don clean PPE.

6.9.18 After disinfection notify the laboratory supervisor and the BSO that the site has been decontaminated.

7.0 ULTRAVIOLET LAMPS

7.1 Exposure to ultraviolet light (UV) may result in serious and painful injury to the eyes or skin depending on the wavelength and intensity of the light and the duration of exposure**.**

7.2 Label all UV light sources conspicuously with the following warning (or equivalent): “Warning – this device produces potentially harmful UV light. Protect eyes and skin from exposure.”

7.3 Ensure that the UV light source is shielded.

7.4 Ensure that appropriate PPE is worn and is sufficient to protect the eyes and skin. PPE should include at least UV resistant face shield, gloves, and lab coat.

7.5 Shielding the equipment or the work area may be warranted.

8.0 ELECTRICAL SAFETY

8.1 Report defects/faults to your supervisor.

8.2 All electrical apparatus must be properly grounded.

8.3 Never remove the ground pin of a 3-pronged plug.

8.4 Inspect electrical cords regularly and have frayed or damaged cords replaced.

8.5 “Piggy-backing” of extension cords is prohibited.

8.6 Never use a power bar beneath workbenches where chemicals are handled.

8.7 **DO NOT** use electric wires as supports and never pull on live wires.

8.8 Ensure that all wires are dry before plugging into circuits.

8.9 **Electrical devices (unless certified explosion-proof) should not be connected outside of the hood** to avoid sparks which may ignite a flammable or explosive chemical.

8.10 Use of Ground Fault Interrupter Circuits (GFCI)is preferable in receptacles located near sinks.

8.11 Circuit breaker panels within laboratories must be easily accessible and clearly marked. Familiarize yourself with their location.

8.12 Only qualified and trained people should repair or modify electrical or electronic equipment.

8.13 Any electrical equipment purchased, regardless of voltage, must be approved as indicated by the presence of a field approval mark from the Canadian Standards Association (CSA), Electrical Safety Authority (ESA), or an equivalent field approval mark acceptable under the Electrical Safety Code i.e. BC Electrical Code Regulation, International Approval Services (IAS) or Intertek Testing Services. The cost of the BC Safety Authority field approvals and modifications, if required, is the responsibility of the acquiring department.

9.0 STATIC ELECTRICITY & SPARKS

9.1 Static electricity and sparks may cause a fire under the right circumstances. Always be conscious of the potential for generating sparks.

9.2 Electrical equipment must have spark protection in areas where there is a danger of fire or explosion.

9.3 Some protection from static electricity and sparks is obtained by proper grounding and bonding of containers and equipment.

9.4 A dry atmosphere promotes the formation of electrical charges.

9.5 Common sources of sparks and static electricity are:

9.5.1 decanting of organic liquids from one metal container to another,

9.5.2 plastic aprons,

9.5.3 metal clamps, nipples or wires used with non-conducting hoses,

9.5.4 gases released quickly from cylinders under high pressure,

9.5.5 switches and thermostats, and

9.5.6 electrical contacts (e.g. light switches and thermocouples, refrigerators) may produce sparks.

10.0 STANDARD OPERATION FOR AUTOCLAVES IN S365

10.1 **Potential Safety** Risks – autoclaves are sterilizers using high pressure and high temperature steam. The potential safety risks for the operators are:

10.1.1 Heat burns -from hot materials and autoclave chamber walls and door.

10.1.2 Steam burns -from residual steam coming out from autoclave and materials on completion of cycle.

10.1.3 Hot fluid scalds- from boiling liquids and spillage in autoclave.

10.1.4 Hand and arm injuries when closing the door.

10.1.5 Body injury if there is an explosion.

10.1.6 Equipment to protect against scalds and burns:

a) Heat-insulating gloves that provide complete coverage of hands and forearms.

b) Closed-toed footwear.

10.2 Operator instructions training

10.2.1 All operators must have successfully completed an authorized training session on the safe operating procedures of this autoclaves. This requirement applies to both new and experienced personnel. A list of authorized users will be kept with the cycle records.

10.3 Before autoclaving the following must be completed

10.3.1 Before turning the autoclave on drain the blue generator by:

a) Opening the tap (labeled #1) at the bottom of the generator,

b) opening the tap (labeled #2) on the side of the autoclave facing the wall to the lab, and

c) allow the water to drain for 5 minutes.

10.3.2 Close the taps that were opened to drain the generator.

10.3.3 Turn on the power supply switch on the wall beside the autoclave (labeled power switch).

10.3.4 Turn on the water supply lever labeled water supply. (it is on when it is parallel with the water supply pipe and off when it is perpendicular to the pipe)

10.3.5 Allow the pressure in the jacket to come up to 15 pounds before starting the autoclave. (about 30 minutes)

10.4 Material Preparation

10.4.1 Ensure that the material is able to be autoclaved. Samples containing solvents or substances that may emit toxic fumes should not be autoclaved.

10.4.2 Glassware must be inspected for cracks prior to autoclaving.

10.4.3 Prepare and package material suitably:

a) Loose dry materials must be wrapped or bagged in steam-penetrable paper or loosely covered with aluminum foil. Wrapping too tightly will impede steam penetration, decreasing efficiency of the process.

b) All containers must be covered by a loosened lid or steam-penetrable bung.

c) Containers of liquid must be a maximum of 2/3 full, with lids loosened.

d) Glassware must be heat-resistant borosilicate.

e) Plastics must be heat-resistant e.g.: polycarbonate (PC), PTFE ("Teflon") and most polypropylene (PP) items.

f) Items or containers must be tagged with autoclave tape to verify sterilization.

g) Loosen all lids to prevent pressure buildup.

h) Add water to containers as appropriate.

10.4.4 Place items in containers to secure and contain spills:

a) items should be placed in a stainless steel or autoclavable plastic container for their stability and ease of handling,

b) place containers of liquid, bags of agar plates, or other materials that may boil over or leak, into a secondary pan in the autoclave,

c) the pan must be large enough to contain a total spill of the contents,

d) bags must not be tightly sealed as steam cannot penetrate, and

e) remove all labels from glassware prior to autoclaving.

10.4.5 Biohazard materials must be labeled as such and secured in containment vessels or autoclavable bags and processed as soon as possible according to requirements for the handling of infectious or biohazard materials.

10.5 Loading Autoclave

10.5.1 Wear heat-insulating gloves, and closed toed shoes.

10.5.2 Place material in autoclave. Do not mix incompatible materials.

10.5.3 Do not overload; leave sufficient room for steam circulation. If necessary, place the container on its side to maximize steam penetration and avoid entrapment of air.

10.5.4 Close and latch door firmly by raising the lid gently till it clicks into place.

10.5.5 **Do Not Let the door slam at the top as it will break the switch located there**.

10.6 Operating Autoclave

10.6.1 Choose appropriate cycle (e.g. liquid, dry unwrapped or wrapped etc.) for the material.

10.6.2 Set appropriate temperature for the cycle (if necessary, 121°C usual temperature).

10.6.3 Press the start button.

10.6.4 Do not attempt to open the door while autoclave is operating.

10.6.5 The manuals for operation of the autoclave are located in the cupboard adjacent to small autoclave and under behind door panel of large autoclave.

10.7 Unloading Autoclave

10.7.1 Wear heat-insulating gloves and closed toed shoes.

10.7.2 Ensure the load is complete.

10.7.3 Wear gloves and stand back from the door as a precaution, carefully crack door open no

more than 1 inch (2.5 cm) to release residual steam and allow pressure within liquids and containers to normalize.

10.7.4 Allow sterilized material to stand for 10 minutes in the chamber. This will allow steam to clear and trapped air to escape from hot liquids, reducing risk to operator.

10.7.5 Do not agitate containers of super-heated liquids or remove caps before unloading.

10.7.6 After removal from the autoclave, place liquid agar in the water bath in the media area that should be turned on before starting the load. This will allow the media to cool to a temp ideal for pouring.

10.8 Equipment Malfunction

10.8.1 If the autoclave does not operate exactly as expected, do not attempt to fix the problem. A notice shall be placed on the autoclave indicating that it is not to be used until the problem is diagnosed and corrected.

10.8.2 Record the problem in the autoclave log book. Contact the Lab Technician to report the problem.

10.8.3 Repair of autoclaves shall be performed by qualified persons only.

10.8.4 All incidents are to be reported to the Lab Technician.

10.8.5 If any injury occurs seek first aid or, if necessary, seek medical assistance by dialing security at 5033.

10.8.6 If clothing is soaked in hot water/steam, remove clothing and cool the injured part in cool water.

10.8.7 Place a notice on the autoclave indicating that it is not to be used until the cause of the incident is determined, procedures enacted to prevent future incidents, and the autoclave is deemed safe for operation.

10.9 Spill clean-up

10.9.1 Spills may occur from a boil over or breakage of containers.

10.9.2 No operation of the autoclave is allowed until the spill is cleaned up.

10.9.3 The operator is responsible for cleanup of spills. Before initiating cleanup, evaluate the situation, shut down the autoclave, remove any contaminated PPE, evacuate the area of personnel using standard exit procedures for 30 minutes to allow aerosols to settle.

10.9.4 Post signage forbidding entry to the area for this period of time.

10.9.5 Exposed personnel should thoroughly wash any exposed areas prior to seeking medical attention if needed.

10.9.6 Supervisor, senior lab staff, or the BSO should be notified of the spill.

10.9.7 After 30 minutes, assemble the spill kit and provided the apparatus has sufficiently cooled, contain the spilled material using paper towel.

10.9.8 Review the MSDS (found on-line using the MSDS online icon on the desk top, if appropriate, to

determine the protective equipment, spill cleanup and disposal protocols that are necessary.

10.9.9 Clean the equipment and work area in order to collect and remove all spilled materials.

10.9.10 Dispose of the waste following the protocol appropriate for the material. If materials have been intermingled, follow the cleanup and disposal protocol for the most hazardous component of the mixture.

10.9.11 Cracked glassware must be disposed of properly in the "Broken Glass" disposal pail.

10.9.12 Report the spill and the clean up to the BSO.

11.0 ETHIDIUM BROMIDE

11.1 Clean Up procedure

11.1.1 Ethidium Bromide is a very common fluorescent intercalating agent used for visualization of nucleic acids. It is sometimes the cause of health and safety concerns for workers charged with using it during the course of their work. Its toxicological properties are not fully determined. There is no evidence supporting or refuting carcinogenicity in humans. Ethidium bromide has been used as an anti-tumorigenic agent in rats and is considered to be non-carcinogenic in rats and mice. It has been found to be mutagenic and genotoxic in various short term in vitro tests such as the Ames test. The precautionary principle suggests that Ethidium bromide be treated as a carcinogen despite the lack of conclusive evidence. Ethidium bromide is toxic with a fairly low LD50 of 50-110mg/kg.

11.1.2 PPE to be worn – nitrile gloves, lab coat, closed toe shoes, UV filtering eyewear (for use with UV light).

11.1.3 Using ethanol, wipe down surfaces with a rag or disposable cloth.

11.1.4 To test for removal, use a UV lamp (black light) to find ethidium bromide that has not been removed. The ethidium bromide will give off a characteristic orange colour. Re-clean areas that were initially missed.

11.1.5 Dispose all wipes and gloves used for removal as chemical waste.

11.1.6 Wash hands and any other area that may have contacted ethidium bromide. Wash lab coat after completion of cleanup.

12.0 CLEANING & MAINTENANCE

12.1 Centrifuges

12.1.1 Maintenance and repairs can only be performed by trained, qualified personnel.

12.2 Gas Chromatographs

12.2.1 The oven must be cooled before maintenance is performed.

12.2.2 The power cord must be disconnected prior to performing maintenance.

12.2.3 Only qualified, trained personnel can perform maintenance and repairs.

12.3 High Performance Liquid Chromatographs

12.3.1 The power cord must be disconnected when performing routine maintenance.

12.3.2 Repairs can only be performed by trained, qualified personnel.

12.4 Autoclave

12.4.1 No person shall operate the autoclave unless the autoclave is in good repair.

12.4.2 Users are not to make repairs.

12.4.3 Report possible malfunctions to the Lab Technician.

13.0 REFERENCES

National Toxicology Program (August 15 2005), Executive Summary Ethidium Bromide: Evidence for Possible Carcinogenic Activity, http://ntp.niehs.nih.gov/?objectid=6F5F63F6-F1F6-975E-79965F7EE68AE7C0. Viewed October 2009

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